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**Nitrogen fertilization effects on soil organic carbon storage and aggregation mechanisms
within continuous corn cropping systems**

by

Kimberly Helen Brown

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Co-majors: Soil Science (Soil Microbiology and Biochemistry);
Environmental Science

Program of Study Committee:
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NOMENCLATURE

SOC	Soil Organic Carbon
LF	Light Fraction
HF	Heavy Fraction
LM	Large Macroaggregates
SM	Small Macroaggregates
Free-m	Free Microaggregates
Inter-m	Inter-aggregate Microaggregates
Free-SC	Free Silt and Clay
POM	Particulate Organic Matter
cPOM	Coarse Particulate Organic Matter
fPOM	Fine Particulate Organic Matter
fiPOM	Fine Intra-aggregate Particulate Organic Matter
Inter-SC	Inter-aggregate Silt and Clay
Intra-SC	Intra-aggregate Silt and Clay
AMF	Arbuscular Mycorrhizal Fungi
AONR	Agronomic Optimum Nitrogen Rate

ABSTRACT

Soils have the potential to store carbon as soil organic carbon (SOC) and reduce atmospheric carbon dioxide (CO₂), a common greenhouse gas. Agriculture can be thought of as the transfer of carbon (C) among different pools, and therefore, proper management can increase SOC storage. Unfortunately, effects on SOC from some of the most common agricultural practices are unknown or difficult to predict. Nitrogen (N) fertilization, for instance, is debated to cause both positive and negative effects on SOC. Effects of N fertilization on SOC levels are determined by the net balance between crop residue inputs (primary source of SOC) and the increase in mineralization. The fate of SOC in these systems is unclear, however, in situations where the soils have reached the upper limit of SOC equilibrium (C saturation). In addition, recent opinions have suggested that by increasing residue quality (lower C:N ratios) through N fertilization, the rate and amount of SOC stabilization will increase. This thesis aims to observe how SOC storage is affected within a continuous corn cropping system in Iowa, USA. Five N application rates were applied for 13 years in Iowa, which increased crop residue quantity and likely residue quality. Stable (protected) SOC pools did not increase, but labile (unprotected) SOC pools increased linearly with crop residue input, thus indicating soils were C saturated based on the two-pool saturation model. Nitrogen application affected the quality (C:N ratio) of coarse particulate organic matter (cPOM), fine particulate organic matter (fPOM), and fine intra-aggregate particulate organic matter (fiPOM), but most strongly affected the cPOM. In fact, cPOM displayed significantly greater regression coefficients when regressed against N application rates than both fPOM and fiPOM, confirming that N fertilization most strongly affected labile pools rather than stable pools. Once degraded to fPOM and fiPOM, C:N ratios become stable and are more reflective of the level of degradation than initial C:N ratios. Along

with a change in residue quality, large macroaggregates displayed increases in the fiPOM:cPOM ratio. Elevated fiPOM:cPOM ratios indicate that microaggregates (inter-m) were more likely to form and store SOC as fiPOM. The increase in inter-m or fiPOM production may have been a result of rapid decomposition of higher quality cPOM. Large macroaggregates did not increase concurrently with the increase in fiPOM:cPOM ratio, and therefore, implies the stabilization did not increase. In addition, microbial biomass, including fungal populations, decreased with crop residue input meaning that microbial biomass or diversity did not influence aggregate dynamics. Not only did macroaggregates create more fiPOM, they also tended to increase the C concentrations of silt and clay (SC) that remained trapped inside macroaggregates. In this study, C saturation prevented new additions of SOC storage, and N fertilization mostly influenced cPOM quality and, in turn, may have shifted aggregation dynamics, allowing for SOC to be stored in more stable forms, such as fiPOM, inter- and intra-SC fractions.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Soil is the largest reservoir of actively cycling C on earth and has the potential to mitigate CO₂ emissions from the atmosphere as SOC. Globally, soils are estimated to store 2344Pg C in the top 3m (Jobbágy and Jackson, 2000). Using soil to reduce atmospheric CO₂ is an attractive option since SOC balances, compared to other global pools, can respond quickly to changes in agricultural management (Lal et al., 1998). Soil organic matter will act as a nutrient source and result in fertile soils, usually corresponding with higher crop yields in agricultural settings (Kazemi et al., 1990; Cruse and Herndl, 2009).

Agriculture can be used to manipulate soil C pools (Lal, 2004). Soils can be managed to store additional C, however, SOC can just as easily be lost by mismanagement. Globally, cropland stores 248Pg C in the top 3m (Jobbágy and Jackson, 2000), and a land-use conversion to cropland likely results in a loss of SOC (Stockmann et al., 2013). For instance, converting grassland to cropland reduces SOC stores by approximately 50% within the first 30 years after conversion (Guo and Gifford, 2002). Others estimate that upon cultivation, soils in the US have lost 30-50% SOC before reaching a new equilibrium (Kucharik et al., 2001). Therefore, it is important to ensure soils in agricultural settings are carefully managed to maintain or increase SOC levels.

Corn is currently planted to over 39 million ha in the US alone (USDA-ERS, 2013) and is easily the most abundant crop. Unfortunately, typical agricultural practices in corn-based systems can result in many alternative fates for SOC, and therefore, SOC balances are highly unknown.

Nitrogen (N) fertilization, for instance, has been debated to have both positive (Buyanovsky and Wagner, 1998) and negative (Khan et al., 2007) effects on SOC balances. Nitrogen fertilizer is a very common soil additive, and is applied to ~95% of the US Corn-Belt corn crop at an average rate of $148 \text{ kg N ha}^{-1} \text{ y}^{-1}$ (USDA-ERS, 2013). Considering the widespread use and the uncertainty of effects on SOC, N fertilization is perhaps the greatest factor affecting SOC in corn-based cropping systems (Cassman, 1999).

Corn residue is incorporated back into the soil as the primary source of SOC. Nitrogen fertilization, therefore, has the potential to increase SOC by increasing crop residue input (Buyanovsky and Wagner, 1998), and due to the flexible nature of plant stoichiometry, N fertilization usually also results in higher residue quality (low C:N ratio; Russell et al., 2009). Microorganisms that decompose crop residue, however, grow with strict stoichiometric requirements (Cleveland and Liptzin, 2007). Since C and N are coupled during decomposition, residues with low C:N ratios (closer to the C:N ratio required by microorganisms) will decompose more rapidly than residues with high C:N ratios (Parton et al., 2007). As a result, the concentration of N in remaining crop residue will increase, thereby lowering the C:N ratio with further decomposition (Waksman, 1924).

The uncertainty of N fertilization effects on SOC arises from the unknown balance between the increase of crop residue and increased SOC mineralization. Russell et al. (2009) showed that

the addition of crop residue input was substantial enough to outweigh the increase in SOC mineralization within continuous corn systems, resulting in a net addition of SOC with N fertilization. It is, however, unknown if all soils will behave similarly even with similar agronomic management, and environmental conditions. A primary concern is that not all soils are able to store the same amount of SOC given equivalent crop residue inputs (Six et al., 2002).

The ultimate fate of crop residue additions is largely determined by antecedent SOC levels, or the proportion of the storage capacity that is already filled (McLaren and Peterson, 1965; Burchill et al., 1981; Hassink and Whitmore, 1997; Stewart et al., 2007). Organic matter additions to soils with low antecedent SOC levels frequently results in an increase in SOC, and is represented in process-based models with linear first-order kinetics so that SOC increases as constant proportion of organic matter inputs (Paustian et al., 1997; Huggins et al., 1998b; Kong et al., 2005). These models suggest an infinite potential for SOC accumulation. In some long-term experiments with high antecedent SOC, however, little-to-no change in C has been detected despite increasing C inputs (Huggins and Fuchs, 1997; Huggins et al., 1998a; Huggins et al., 1998b; Reicosky et al., 2002). These results led to the concept that soils have a finite capacity to store SOC termed 'C saturation' (Six et al., 2002; Stewart et al., 2007).

Two models have been developed to describe C saturation dynamics (Stewart et al., 2007). A one-pool whole soil C saturation model suggests soils have a finite C storage capacity and the efficiency of C storage decreases with increasing input. Alternatively, a two-pool model suggests soils contain two distinct C pools: a stable (protected) pool that saturates and a labile (unprotected) pool that does not saturate. The stable pool includes SOC fractions that are

physico-chemically protected from mineralization through mineral-association (Sollins et al., 1996) and microaggregation (Jastrow et al., 1996; Six et al., 1998; Gale et al., 2000; von Lutzow et al., 2006). Particulate organic matter (POM) that is included in the labile pool is not occluded within microaggregates and has no long-term physical protection. Transfer efficiency of decomposition products from the labile pool to the stable pool decreases in proportion to saturation deficit in the stable pool (McLaren and Peterson, 1965; Burchill et al., 1981). The labile pool will increase exponentially as the stable pool reaches saturation, and will increase without limit. Although there is no limit to accumulation, the rate of decomposition is faster for the labile pool making soil unlikely to accumulate C in large excess. This process is observed when nutrient availability increases as labile SOC accumulates due to stable pool saturation (Stewart et al., 2007; Gulde et al., 2008; Castellano et al., 2012).

Similar to the two-pool saturation model which suggests antecedent C levels of the protected pool affect the partitioning of new organic matter inputs among stable and labile pools, N fertilizer can also affect the stabilization and mineralization of new organic matter inputs. By increasing residue quality, N fertilizer can influence SOC storage dynamics by altering microorganism activity (Cotrufo et al., 2013), diversity (Ramirez et al., 2012), biomass (Soderstrom et al., 1983; Nohrstedt et al., 1989; Liebig et al., 2002), and carbon use efficiencies (Manzoni et al., 2010). Microbial byproducts, which act as binding agents within aggregates, have a profound impact on the formation and stabilization of aggregates, making microbial activity a major component of aggregation mechanisms (Oades, 1993; Golchin et al., 1994).

Soil aggregates physically protect SOC from rapid decomposition by limiting microbial access (Tisdall and Oades, 1982) and by decreasing oxygen and water influx (Sexstone et al., 1985; Prove et al., 1990; Smucker et al., 2007), which are both essential factors involved in the decomposition process. Aggregates vary in size, and are categorized into different size classes based on diameter. The three aggregate size classes typically defined are large macroaggregates (LM), small macroaggregates (SM), and microaggregates (free-m). Diameters of these size classes are $> 2000\mu\text{m}$, $250\text{-}2000\mu\text{m}$, and $53\text{-}250\mu\text{m}$ (Márquez et al., 2004), respectively. Any particle $< 53\mu\text{m}$ is considered free silt and clay (free-SC). Due to small stature and micro-sized pores, microaggregates tend to be more stable than macroaggregates and are key factors in storing SOC for the long-term (Oades and Waters, 1991; Jastrow and Miller, 1998; Six et al., 1998; Gale et al., 2000).

The current model of soil aggregate formation says microaggregates form within macroaggregates, contingent on the macroaggregates being stabilized long enough to allow microaggregate formation (Oades, 1984; Elliott and Coleman, 1988). Macroaggregates are formed around freshly deposited organic residue by the initiation of microbial decomposition (Golchin et al., 1994; Jastrow, 1996; Six et al., 1999a). Continued decomposition of organic material inside the aggregate slowly creates microaggregates within macroaggregates (inter-microaggregates), encrusting any remaining POM.

Macroaggregates routinely form, disperse, and re-form over time. The rate at which this occurs is termed the macroaggregate turnover rate, and is highly influenced by the level of macroaggregate stability (Six et al., 2000). Overtime, POM fractions within macroaggregates

decrease in size as coarse POM (cPOM) decomposes to fine or fine intra-aggregate POM (fiPOM or fiPOM; Guggenberger et al., 1994), therefore, the fiPOM:cPOM ratio represents a proxy of macroaggregate turnover rate (Six et al., 2000). Higher ratios suggest fiPOM levels are elevated in relation to cPOM and result from increased aggregate stabilization or slow turnover rates. Considering the formation of microaggregates is essential in long-term SOC storage, stabilization of macroaggregates and a slow turnover rate become key factors (Oades & Waters, 1991; Jastrow and Miller, 1998; Six et al., 1998; Gale et al., 2000).

Microorganisms play a key role in aggregation by excreting a byproduct that aids in aggregate formation and stabilization during decomposition (Oades, 1993; Golchin et al., 1994). For instance, because high quality residues are rich in N content, these residues usually experience rapid initial decomposition (Hobbie, 2005). Microbes, no longer N limited, decompose organic material with greater microbial use efficiency (Manzoni et al., 2010), resulting in a slowing of SOC mineralization and more residue mass remaining in later stages of the decomposition process (Fog, 1988). Considering high quality residues may result in a greater amount of high quality cPOM inside newly formed macroaggregates, cPOM may decompose more quickly to fiPOM, meaning that microaggregates would also form quickly. Under these conditions, SOC may be more likely stored in stable fractions, such as within microaggregates, even under faster macroaggregate turnover rates.

Recent research found that low quality corn residue additions increase macroaggregate formation (Chivenge et al., 2011). Research by Puttaso et al. (2012) took this concept further and also showed that high quality residues were responsible for microaggregate formation due to the

increase in microbial activity (Puttaso et al., 2012). Together, these studies suggest that low quality residues may only affect macroaggregate formation, whereas high quality residues may affect microaggregate formation.

Clearly, the effects of N fertilization on SOC balances are uncertain, especially when soils are C saturated, and could result in many different alternatives. With further research, the fate of SOC will be more predictable, and by discovering the fractions that store large sums of SOC, mechanisms responsible for increases in SOC will be uncovered. By discovering and implementing agronomic practices that tend to incorporate SOC as stable forms, agriculture may have the potential to increase SOC worldwide (Lal, 2004).

Objectives and Organization

This thesis presents a study in Chapter 2 that aims to observe the interactions of N fertilization and crop residue inputs on the dynamics of SOC storage. The study site was a continuous corn cropping system in Iowa, USA. Soils receiving large amounts of N fertilization and crop residue input were likely C saturated and therefore, created a unique study site, where incoming C was shunted to an unprotected pool. The overall conclusions from this study are summarized in Chapter 3.

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CHAPTER 2. NITROGEN FERTILIZATION EFFECTS ON SOIL ORGANIC CARBON STORAGE AND AGGREGATION MECHANISMS WITHIN CONTINUOUS CORN CROPPING SYSTEMS

A paper to be submitted to the journal *Global Change Biology*

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Abstract

Agriculture transfers carbon (C) among different global pools, and therefore, proper agricultural management can increase soil organic carbon (SOC) storage. Effects on SOC of some of the most common agricultural practices are unknown or difficult to predict across different soil types or cropping systems. Nitrogen (N) fertilization is a common practice with corn-based systems and can cause both positive and negative effects on SOC balances. Nitrogen fertilization can increase SOC by increasing crop residue input (primary source of SOC), and it can also decrease SOC by increasing mineralization. It is unclear, however, how soil C saturation affects SOC storage in these systems. In addition, recent opinions have suggested that by increasing residue quality (lower C:N ratios) through N fertilization, more SOC will be stabilized. To observe these

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possible effects and how they interact, five N rates were applied to a continuous corn system for 13 years in Iowa, USA. Crop residue quantity ranged from 3.60 Mg dry matter ha⁻¹y⁻¹ without N fertilization to 9.94 Mg dry matter ha⁻¹y⁻¹ with the highest N application rate, creating a residue input gradient. Stable (protected) SOC pools did not increase, but labile (unprotected) SOC pools increased linearly with crop residue input, indicating soils were C saturated based on the two-pool saturation model. Nitrogen application affected the quality of coarse particulate organic matter (cPOM), fine particulate organic matter (fPOM), and fine intra-aggregate particulate organic matter (fiPOM), but most strongly affected cPOM. In fact, cPOM displayed significantly different regression coefficients than both fPOM and fiPOM, implying that N fertilization most strongly affected cPOM. Along with a change in cPOM quality, large macroaggregates displayed increases in the fiPOM:cPOM ratio. Elevated fiPOM:cPOM ratios indicate that microaggregates (inter-m) were more likely to form and store SOC as fiPOM. The increase in inter-m or fiPOM production may have been a result of rapid decomposition of higher quality cPOM. Large macroaggregates did not increase concurrently with the increase in fiPOM:cPOM ratio, and therefore, implies the stabilization did not increase. In addition, microbial biomass, including fungal populations, decreased with crop residue input meaning that microbial biomass or diversity did not influence aggregate dynamics. Not only did macroaggregates create more fiPOM, they also tended to increase the C concentrations of silt and clay (SC) that remained trapped inside macroaggregates. In this study, C saturation prevented new additions of SOC, however, N fertilization most strongly affected cPOM and may have shifted aggregation dynamics, allowing for SOC to be stored in more stable soil fractions, such as fiPOM and as mineral-associated SOC.

Keywords: soil organic carbon storage; nitrogen fertilization; aggregates; microbial diversity

Abbreviations: N, nitrogen; C, Carbon; CO₂, carbon dioxide; SOC, soil organic carbon; LF, light fraction; HF, heavy fraction; LM, large macroaggregates; SM, small macroaggregates; Inter-m, inter-aggregate microaggregates; Free-m, free microaggregates; Free-SC, free silt and clay; POM, particulate organic matter; cPOM, coarse particulate organic matter; fPOM, fine particulate organic matter; fiPOM, fine intra-aggregate particulate organic matter; Inter-SC, inter-aggregate silt and clay; Intra-SC, intra-aggregate silt and clay; AMF, arbuscular mycorrhizal fungi; AONR, agronomic optimum nitrogen rate

Introduction

Corn cropping systems currently cover over 39 million ha in the US (USDA-ERS, 2013) and represent an enormous stock of actively managed SOC. The status of SOC balances in US Corn-Belt is currently uncertain. Positive, negative, and neutral balances are reported (Adviento-Borbe et al., 2007; Khan et al., 2007; David et al., 2009; Senthilkumar et al., 2009). Applied to ~95% of the US Corn-Belt corn crop at an average rate of 148 kg ha⁻¹ y⁻¹ (USDA-ERS, 2013), N fertilizer is perhaps the greatest factor affecting SOC balances in corn-based cropping systems (Cassman, 1999). Nitrogen fertilizer can increase SOC by increasing organic matter (crop residue) inputs to the soil. Alternatively, N fertilizer can decrease SOC by increasing C mineralization. Generally, inorganic N fertilizer increases both organic matter inputs and C mineralization, but the net effect of N fertilizer on SOC is positive when N fertilizer is not applied in large excess (Russell et al., 2009; David et al., 2010; Powlson et al., 2010).

In the absence of inorganic N fertilizer, a positive effect of organic matter inputs on SOC is frequently observed and represented in process-based models with linear first-order kinetics so that SOC increases in constant proportion to organic matter inputs (Paustian et al., 1997a; Huggins et al., 1998b; Kong et al., 2005). These models suggest an infinite potential for SOC accumulation. Some long-term experiments with high initial SOC, however, show no differences in soil C having been detected across treatments despite a large range of C inputs (Huggins and Fuchs, 1997; Huggins et al., 1998a; Huggins et al., 1998b; Reicosky et al., 2002). These results led to the concept that soils have a finite capacity to store soil organic C (SOC) termed ‘C saturation’ (Six et al., 2002; Stewart et al., 2007).

Two models have been developed to describe C saturation dynamics (Stewart et al., 2007). A one-pool whole soil C saturation model suggests soils have a finite C storage capacity and the efficiency of C storage decreases with increasing input. Alternatively, a two-pool model suggests soils contain two distinct C pools: a stable (protected) pool that saturates and a labile (unprotected) pool that does not saturate. The stable pool includes SOC fractions that are physico-chemically protected from mineralization through mineral-association (Sollins et al., 1996) and microaggregation (Jastrow et al., 1996; Six et al., 1998; Gale et al., 2000; von Lützow et al., 2006). The labile pool includes organic matter that is not mineral-associated or occluded within microaggregates and thus has no physical limit on accumulation. Decomposition rates, however, increase for the labile pool, making it difficult to accumulate in large excess. In the two-pool model, the transfer efficiency of decomposition products from the labile pool to the stable pool decreases in proportion to amount of C in the stable pool (McLaren and Peterson,

1965; Burchill et al., 1981); organic matter inputs that are not transferred to the stable pool accumulate in the labile pool or are mineralized (Stewart et al., 2007; Gulde et al., 2008; Castellano et al., 2012).

Similar to the two-pool saturation model which suggests antecedent C levels of the protected pool (i.e., saturation deficit) affect the partitioning of new organic matter inputs among stable and labile pools, inorganic N fertilizer can also affect the stabilization and mineralization of new organic matter inputs. Nitrogen fertilizer can affect aggregation processes by altering several aggregate forming and stabilizing mechanisms, including microbial diversity (Ramirez et al., 2012), biomass (Soderstrom et al., 1983; Nohrstedt et al., 1989; Liebig et al., 2002), and carbon use efficiencies (Manzoni et al., 2010).

Microorganisms play a key role in aggregation by excreting a byproduct that aids in aggregate formation and stabilization during decomposition (Oades, 1993; Golchin et al., 1994). For instance, because high quality residues are rich in N content, these residues usually experience rapid initial decomposition (Hobbie, 2005). Microbes, no longer N limited, decompose organic material with greater carbon use efficiency (Manzoni et al., 2010), resulting in a slowing of decomposition and more residue mass remaining in later stages of the decomposition process (Fog, 1988). Considering high quality residues may result in a greater amount of high quality cPOM inside newly formed macroaggregates, cPOM may decompose more quickly to fiPOM, meaning that microaggregates would also form quickly. Under these conditions, SOC may be more likely stored in stable fractions, such as within microaggregates, even without an increase in macroaggregate stabilization.

Together, these data suggest C saturation status and N fertilization interact to affect SOC storage. The relative importance of these factors as well as the balance between positive and negative effects of N fertilizer on SOC stabilization is unknown, particularly with direct reference to agronomic optimum N inputs (i.e, N maximizes production, but does not exceed crop demand). Our objectives were to evaluate C saturation concepts as well as the net effect of N fertilizer on SOC storage through its effects on crop residue inputs and SOC dynamics. To accomplish these objectives we indentified a continuous corn cropping system that has received one of five N fertilizer rates (0-269 kg N ha⁻¹) annually for 13 years while all other nutrients and pH are managed for agronomic optimum. The site was well suited to address our objectives because it included three N rates that were insufficient to maximize NPP, one rate within 7 kg N ha⁻¹ y⁻¹ of agronomic optimum N input, and one rate that provided a large excess of inorganic N beyond crop demand (>60 kg N ha⁻¹ y⁻¹ excess). Across these N input rates, organic matter inputs varied from 2.88-10.24 Mg dry matter ha⁻¹ y⁻¹.

Methods

Site Description and Experimental Design

The study site was established in 1999 on the Iowa State University Research Farm (42°0'38" N, 93°47'19" W). Research plots were maintained in continuous-corn with fall disk-chisel plow tillage. Plots contained six corn rows in a 4.5m by 15.25m area. Five N application rates were applied in a randomized complete block design with four replications. Nitrogen was applied

annually in mid-May, usually 5-10 days after planting. Application rates included 0, 67, 135, 202, 269 kg N ha⁻¹ y⁻¹ and was applied as 32% urea and ammonium nitrate (UAN) solution in subsurface bands. Band spacing was 1.5m. After grain harvest, crop residue was tilled into the soil, creating a carbon input gradient across treatments. All nutrients, other than N, and pH were maintained for agronomic optimum. Agronomic optimum N rate (AONR; Gentry et al., 2013) was estimated as the join point (Anderson and Nelson, 1975) of a fitted quadratic-plateau model of the yield data (1999-2011).

Crop Residue Input

Crop residue input was estimated from the average annual grain yield (1999-2011) by assuming a 0.5 harvest index, which evidence suggests is accurate and does not vary significantly with N rate (Russell et al., 2009; Lorenz et al., 2010). Belowground residue was not included in our estimations of residue input because total residue has been shown to be dominated by the aboveground growth. Russell et al. (2009) studied the effects of N fertilization on corn grown across similar N gradients and soil types and found that harvest index ranged from 0.45 to 0.51 and did not vary with N application. In addition, belowground productivity was not influenced by N application. Belowground productivity accounted for only 6-22% of the total productivity and the root:shoot ratio ranged from 0.11 to 0.12. Despite the harvest index and root:shoot ratios not being influenced by N application, total productivity did increase with N application (Russell et al., 2009).

Soil Sampling and General Soil Property Analysis

In July of 2011, three soil cores were taken to 30cm depth and combined from each plot. Soil samples were only taken within the three center rows of the six-row plots to avoid border rows and any possible N/crop residue movement across plots. These cores were passed through an 8mm sieve upon return to the lab and then air-dried. Bulk density was estimated from these cores. Seven additional soil cores were taken from similar locations of the center plot rows and combined. These cores were air-dried and a portion was ground for quantification of plant-available phosphorus (Bray-P1) and pH (1:1 soil: water ratio). Bray-P1 analysis (Bray and Kurtz, 1945) was conducted at the Iowa State University Soil and Plant Analysis Laboratory. A hydrochloric acid-ammonium fluoride (HCl-NH₄F) extraction method was used to extract plant-available phosphorus, which was quantified by a colometric method. The portion of soil that was not ground was used for textural analysis with the method of Kettler et al. (2001).

Fractionation Procedure

The fractionation procedure (Figure 1) follows that of Gulde et al. (2008), with a slight modification in the light fraction (LF) removal. Instead of removing the LF after wet-sieving, the LF was removed during wet-sieving. This procedure isolated water-stable aggregates by wet-sieving, and then further fractionated large and small macroaggregates to isolate POM and silt and clay fractions within.

Water-stable Aggregate Isolation and Light Fraction Removal

Water-stable aggregate classes were isolated by wet-sieving (Elliot, 1986). Isolated fractions included large macroaggregates (LM), small macroaggregates (SM), free microaggregates (Free-m), and free silt and clay (Free-SC). Diameters of these fractions are $>2000\mu\text{m}$, $250\text{-}2000\mu\text{m}$, $53\text{-}250\mu\text{m}$, and $<53\mu\text{m}$, respectively (Màrquez et al., 2004). Briefly, about 100g of air-dry soil was slaked for five minutes prior to wet-sieving. After slaking, the soil was agitated over a $2000\mu\text{m}$ sieve by re-submerging the sieve into water 50 times over two minutes. The LF, or any floating residue, was removed at this step with a net, and any soil remaining on the sieve was washed into a drying pan and dried at 65°C overnight. The procedure was repeated by agitating the remaining soil over a $250\mu\text{m}$ and a $53\mu\text{m}$ sieve by the same procedure. Return in soil mass after the wet-sieving process averaged 99.22% (± 0.63) and ranged from $99.12\text{-}100.49\%$. Percent return higher than 100% can result if soils are not fully oven-dried before being weighed, but this only occurred $\sim 8\%$ of the time. All aggregate fractions were corrected for sand and LF (Elliot et al., 1991). Total C and N were analyzed for each isolated fraction by dry-combustion.

Large and Small Macroaggregate Fractionation

We isolated microaggregates within macroaggregates (i.e., inter-microaggregates (inter-m)), with the protocol outlined by Six et al., (2000). Briefly, $10\text{-}15\text{g}$ of macroaggregates (sometimes $\sim 5\text{g}$ for large macroaggregates due to limited sample size) were slaked for $15\text{-}20$ min before being exposed to minor abrasion mechanical shaking in running water with 50 glass beads. This was done over a $250\mu\text{m}$ sieve to collect coarse particulate organic matter (cPOM) that was within

macroaggregates. All soil $<250\mu\text{m}$ ran through the sieve and was collected in a receptacle equipped with a $53\mu\text{m}$ sieve. The wet-sieving procedure, as mentioned above, was done on the soil collected on this sieve. This procedure separated the inter-microaggregates from the silt and clay that was within macroaggregates, but not within microaggregates (i.e., inter-silt and clay (inter-SC)). All fractions isolated with this procedure were oven-dried at 60°C .

Inter-microaggregate Fractionation

To disperse inter-microaggregates, which consist of fine intra-aggregate particulate organic matter (fiPOM) encrusted with silt and clay (i.e., intra-silt and clay (intra-SC)), samples were shaken in 0.5% sodium hexametaphosphate ($(\text{NaPO}_3)_6$) at a 1:3 ratio (soil:liquid; v,v) overnight. Dispersed samples were washed through a $53\mu\text{m}$ sieve to separate fiPOM from intra-SC. All fractions isolated with this procedure were oven-dried at 60°C .

Density Flotations

Sodium polytungstate ($\text{Na}_6[\text{H}_2\text{W}_{12}\text{O}_{40}]$) at the density of 2.3 g cm^{-3} was used to remove sand contamination from the cPOM (sand $>250\mu\text{m}$) and fiPOM ($53\mu\text{m} < \text{sand} < 250\mu\text{m}$) fractions. A density flotation at 1.85 g cm^{-3} was also used to remove fPOM from the Inter-m fraction before dispersion. Briefly, samples were added to sodium polytungstate at the desired density with a 1:3 ratio (soil:liquid; v,v). Suspended soil within the solution was gently stirred once to ensure light material trapped below dense material was released and left to settle overnight. After settling overnight, floating material was aspirated from the surface by vacuum filtration using a

20 μ m nylon mesh filter. Any remaining sodium polytungstate was washed off with deionized water 7-10 times before oven-drying at 60°C. Once cPOM and fiPOM fractions were isolated from each macroaggregate class, macroaggregate turnover was estimated by the fiPOM:cPOM ratio (Six et al., 2000).

Carbon and Nitrogen Measurement and Interpretation

Carbon and nitrogen content was determined with a dry combustion elemental analyzer for the whole soil, water-stable aggregates, free-SC, cPOM, fPOM, fiPOM, Inter-SC, and Intra-SC. We interpreted C concentrations for each fraction as a percent of whole soil C (g fraction C kg⁻¹ whole soil). When evaluating C saturation dynamics, we also interpreted C concentrations within individual free-SC, inter-SC, and intra-SC fractions (g fraction C g⁻¹ fraction) because silt and clay, unlike aggregates, do not have the ability to increase in proportion of the whole soil. Instead, silt and clay have a finite capacity to stabilize C (Hassink and Whitmore, 1997).

Soil organic C associated with cPOM and fPOM were considered unprotected or labile since they are located inside macroaggregates, which are destroyed upon relatively minor soil disruption (Elliot, 1986; Six et al., 2000). If not incorporated into microaggregates, these POM fractions lack long-term physical protection. Soil organic C associated with free-m, free-SC, fiPOM, inter-SC, and intra-SC were considered protected or stable pools. We quantified the amount of unprotected carbon by summing C from cPOM and fPOM located within large and small macroaggregates. The remaining C within the macroaggregates, along with the C from free-m and free-SC, represented the protected pool.

Microbial Sampling and Analysis

Fatty Acid Methyl Ester (FAME) analysis was used to observe the diversity of the microbial community among treatments. In 2012, soil samples were taken from the field site in a similar fashion as the prior year except that samples were sent through a 4mm sieve upon returning to the laboratory and then immediately frozen. Briefly, 10ml of 0.2M potassium hydroxide(KOH):methanol(MeOH) (1:1, v/v) was added to 5g soil samples and placed into a water bath at 37°C for 1 hour, shaking occasionally. Extracted fatty acid methyl esters were dissolved (after being dried under N₂ gas) in hexane (C₆H₁₄) containing a fatty acid internal standard C19:0 before inserting 50µl aliquot to the conical gas chromatograph vial. Biomarkers for bacteria included, iC14:0, iC15:0, aC15:0, C15:0, iC16:0, i10MeC17:0, C17:1unknown, iC17:0, aC17:0, C17:1c9, C17:0, 10MeC19:0, cyC19(9,10), and cyC19(11,12) (Frostegård and Bååth, 1996; DeGrood et al., 2005; McKinley et al., 2005). Biomarkers for fungi differentiated between saprophytic fungi and AMF. Biomarkers for saprophytic fungi included, C18:2c9,12 and C18:1c9 (Frostegård and Bååth, 1996; Stahl and Klug, 1996). The biomarker for AMF was C16:1c11 (Olsson and Johansen, 2000). Bacteria:fungi ratio was calculated by dividing bacterial biomass by the sum of AMF and saprophytic fungi biomass. Total microbial biomass was estimated by summing the biomass of bacteria, AMF, and saprophytic fungi.

Statistical Analysis

All statistical tests were performed with SAS 9.2 (SAS Institute, Cary, NC). Large and small macroaggregates were fractionated into three types of POM (cPOM, fPOM, fiPOM), and although there are documented distinctive characteristics among POM fractions (i.e. cPOM vs fiPOM; Six et al., 2000), it is not clear if the originating aggregate adds distinctiveness (i.e. large macroaggregate cPOM vs small macroaggregate cPOM). Large and small macroaggregates were also fractionated into two types of silt and clay fractions, and similarly, it is not clear if there is distinctiveness between fractions (i.e. inter- vs intra-SC) or among originating aggregates (i.e. large macroaggregate inter-SC vs small macroaggregate inter-SC). To test these premises statistically, an ANOVA model that included the fraction and the originating aggregate as fixed categorical factors was used in SAS with the MIXED procedure. In both cases, statistical results showed distinctiveness was present between fractions but the originating aggregate did not matter. Therefore, results from different aggregate classes were combined for each fraction (N=40).

Relationships between the dependent variables (C and N concentrations, C:N ratios and microbial biomass) and independent variables (N fertilizer rate and crop residue input) were analyzed with linear and nonlinear regression models. The best fit equation (linear vs. non-linear) was based on the lowest sum of squares error. To test for linear relationships as well as significant differences among regression coefficients or slopes the SAS REG procedure was used. To test for nonlinear exponential relationships, the SAS NLIN procedure was used. The SAS NLIN procedure was also used to fit a quadratic model to the relationship among yield and

N fertilizer rate as is the standard procedure in soil fertility literature (Cassman and Plant, 1992; Dobermann et al., 2000). The R values were calculated for nonlinear relationships with the following equation: $R = \sqrt{1 - SSE/SST}$, where SSE is the sum of squares error and the SST is the sum of squares of the corrected total (Kvalseth, 1985). It is important to note that all regression analyses were computed with all data points (20 plots); however, figures display only the means and standard errors from each of the five N rates for visual clarity.

Results

General Soil Properties

Bulk density and soil texture did not significantly differ among N applications (Table 2.1). Among all plots, mean (\pm standard error) plant available (Bray) phosphorus was 34.06 ppm (± 4.47). Although plant available phosphorus decreased with N application ($P=0.0285$), all plots were above the agronomic optimum phosphorus soil level for corn production in Iowa (Sawyer et al., 2002; A. Mallarino, personal communication, June 2013). Soil pH averaged 6.03 (± 0.12) among all plots. Although pH decreased with N application ($P=0.0119$), values fluctuated around a pH of 6.0 and were within the agronomic optimum for the Clarion soil series (Sawyer et al., 2002). These relationships for phosphorus and pH reflect changes since the last nutrient/lime additions (Fall, 2011) rather than differences that existed throughout the duration of the N rate experiment (1999-present).

Crop Residue Input

Average annual aboveground crop residue production ranged from 3.60-9.94 Mg dry matter ha⁻¹ y⁻¹, and significantly increased with N application (Table 2.1, Figure 2.2a). The relationship between crop residue production and N rate was fit to a quadratic model with residue production reaching a plateau at 202 kg N ha⁻¹ y⁻¹. Based on yield data from 1999-2011, the AONR was 208.4 kg N ha⁻¹ with a yield response of 9.77 Mg dry matter ha⁻¹.

Soil Carbon and Nitrogen Fractions

Total SOC ranged from 16.22-24.49 g C kg⁻¹ soil among individual plots and did not increase with N rate or crop residue input (Table 2.1, Figure 2.2b,c). The whole soil C:N ratio did not differ among N fertilization rates (Figure 2.3), however, the C:N ratio for unprotected organic matter decreased with N application ($P < 0.0001$), and ranged from 14.83-24.75. If unprotected organic matter were broken down into separate POM fractions, C:N ratios ranged from 14.67-25.19, 14.41-28.37, and 13.87-16.78 for cPOM, fPOM, and fiPOM, respectively (Figure 2.3). Although all of these relationships were significant, the regression coefficients or slopes significantly differed among these POM fractions. The regression coefficient for cPOM was greater than both fPOM ($P = 0.0027$) and fiPOM ($P < 0.0001$), but regression coefficients did not differ between fPOM and fiPOM. In addition, average POM C:N ratios decreased in the following order: cPOM > fPOM, > fiPOM. Because the effect of N application rate on C:N ratios was limited to the POM fraction, we focus all further analyses on relationships with crop residue input rather than N application rate.

The proportions of water-stable aggregate classes did not differ across crop residue inputs. Aggregate class averages were 2.14 (± 0.38), 28.67 (± 0.96), and 22.89 (± 0.93) g 100 g⁻¹ soil for LM, SM, and free-m, respectively. The mass of free-SC averaged 8.20 (± 0.29) g 100 g⁻¹ soil and also did not differ across crop residue inputs (Table 2.2).

The protected SOC pool, on a whole soil basis (mass of fraction in whole soil*fraction C concentration), did not significantly differ for any fraction across crop residue inputs (Figure 2.4). Protected classes averaged 0.49 (± 0.11), 5.13 (± 0.33), 4.48 (± 0.24), and 1.78 (± 0.06) g C kg⁻¹ whole soil for LM, SM, free-m, and free-SC, respectively. Note that cPOM, and fPOM are not included in protected fraction calculations, and also that all fractions are corrected for sand and LF. In addition, average protected SOC stored in each fraction decreased in the following order: SM > free-m > free-SC > LM. The unprotected SOC pool (sum of cPOM and fPOM), also on a whole soil basis, increased with crop residue input (Figure 2.5). Among individual plots, unprotected C ranged from 0.2111-0.5360 g C kg⁻¹ whole soil. After fractionating the soil into 12 fractions and summing the C from each, the calculated value for whole soil C was very similar to the actual value of whole soil C. Calculated whole soil C values averaged 102.32% (± 5.06) of the actual whole soil C values. Unlike the fiPOM:cPOM ratio for SM, the fiPOM:cPOM ratio for LM did increase with crop residue input (Figure 2.6). Among individual plots, LM fiPOM:cPOM ratio ranged from 0.1451-1.2516.

There were no significant differences in C concentrations within free-SC fractions across crop residue inputs, and averaged 21.72 g C kg⁻¹ free-SC (± 0.2104). Carbon concentration of silt and

clay fractions held within macroaggregates (inter- and intra-SC), however, increased with crop residue input (Figure 2.7). Among individual plots, C concentrations for inter- and intra-SC ranged from 22.06-28.28 and 15.46-31.98 g C kg⁻¹ SC, respectively.

Microbial Diversity

Lipid abundance, among all plots, ranged from 29.65-50.50, 6.84-29.40, and 20.05-34.89 nmol g⁻¹ soil for bacteria, AMF, and saprophytic fungi, respectively (Figure 2.8b). All trends for microbial populations were significant, but responded in different ways. Bacteria increased, but both AMF and saprophytic fungi decreased with crop residue input. The increase in bacterial populations was not enough to compensate for the decrease in fungal populations, however, and total microbial biomass decreased with crop residue input (Figure 2.8a). Total microbial biomass ranged from 66.4-98.5 nmol g⁻¹ soil. When AMF and saprophytic fungi are summed to represent total fungi, the bacteria:fungi ratio increased with crop residue input (Figure 2.8c). The bacteria:fungi ratio ranged from 0.5360-1.5399.

Discussion

Soil Organic Carbon Storage

Despite 13 years of residue inputs ranging from 3.60-9.94 Mg dry matter ha⁻¹ y⁻¹, we found no differences in SOC concentrations across N application rates or crop residue input rates. These results suggest that either positive (crop residue inputs) and negative (C mineralization) effects

of N fertilization were equivalent (Russell et al., 2009), or that soils were C saturated. Although we cannot say for certain if soils were C saturated since there was no sampling at the initiation of the experiment, we can relate our results to the two-pool saturation model (Stewart et al., 2007). In this context, protected pools appeared saturated, with no differences in SOC concentrations among crop residue inputs. In addition, differences in the amount of C contributed by each fraction to the protected pool were more reflective of mass proportion of the whole soil than C concentrations since these fractions had very similar C concentrations. The unprotected pool, however, was positively correlated with crop residue inputs, indicating that any additional C input was likely shunted to the unprotected pool since protected pools, representing a large sum of total SOC, were already saturated. The protected pool held 97.10% of the whole soil C, and among the protected pool, SM, free-m, free-SC, and LM fractions held 43.20%, 37.74%, 14.97%, and 4.10%, respectively. The unprotected pool, depending on the amount of crop residue input, held only 1.93-3.35% of the total SOC. Given the fast rate of decomposition in the unprotected pool, excess SOC accumulation in this pool is unlikely. Also, since the unprotected pool contributed a very small proportion to the whole soil C, the differences in C seen in the unprotected pool were not enough to result in significant changes in whole soil C.

Nitrogen Application Effect

Due to the flexible stoichiometry of plants, corn residue C:N ratio decreases with increasing N application. Although we did not measure the residue C:N ratio of corn residue at our site, Russell et al. (2009) found that corn residue C:N ratios in Iowa decreased by >50% with increasing N application. Since POM directly originates from crop residue inputs, it was not

surprising that POM fractions showed decreasing C:N ratios with increasing N application. It was surprising, however, that different POM fractions were affected at different degrees. Coarse POM had the steepest regression coefficient or slope with N rate and was thus most strongly affected by N application. The range of C:N ratios across N application rates narrowed with each step of decomposition from cPOM to fPOM and fiPOM. Average C:N ratios ranged by 4.78 units among cPOM samples, 1.69 units once cPOM was degraded to fPOM, and 0.51 units once fPOM was degraded to fiPOM. These results show that if N application were to influence decomposition rates due to changes in POM quality, it would most strongly affect cPOM. In addition, cPOM showed the highest average C:N ratio, followed by fPOM, and then fiPOM. Therefore, microorganisms lower the C:N ratio with each step of degradation. Without N fertilization influencing the quality of cPOM, C:N ratios would drop approximately 2.28 units when cPOM was degraded to fPOM and 2.92 units when fPOM was degraded to fiPOM. Once degraded past cPOM or the level at which N application effects residue quality, POM C:N ratios are more reflective of the level of degradation than by initial residue quality.

Aggregate Dynamics

Although there were no changes in SOC levels, the fiPOM:cPOM ratio of LM increased with increasing crop residue input, implying that SOC was being stored as more stable forms, such as fiPOM. The fiPOM:cPOM ratio is a proxy of aggregate turnover rate, and aggregates with high fiPOM:cPOM ratios usually have slower turnover rates and are better stabilized, which allows for the formation of inter-m. Over time, this may slowly increase SOC levels by increasing the proportion of LM and free-m. In our results, however, an increase in the fiPOM:cPOM ratio is

not accompanied with an increase in LM or free-m. Since LM represented a very small proportion of the total soil (1.43-3.31 g 100 g⁻¹ soil), additions of free-m were also likely very small. Considering the free-m represent a large proportion of the total soil in comparison (21.23-24.79 g 100 g⁻¹ soil), small additions of free-m may have been negligible or difficult to detect. Given more time, or a soil that has a larger proportion LM, this response may be more apparent. The addition of stabilized LM would have also been small, but should have been much easier to detect due to the small proportion of initial LM. This implies that the LM did not experience an increase in stabilization, but did experience an increase in inter-m formation.

Interestingly, the increase in cPOM quality may have contributed to the increase in inter-m formation, without the stabilization of LM. Decomposition of residues with high quality (low C:N ratios) are rich in N and, therefore, decompose quickly at first (Hobbie, 2005). This may mean that the quick decomposition of cPOM allows quick formation of inter-m and storage of SOC as fiPOM, and suggested by our results, may even do so without an increase in LM stabilization.

Microbial Diversity and Biomass

Given that fungal biomass decreased with crop residue input, it appears that fungi did not contribute to aggregation dynamics. This comes with surprise since fungal dominated soils are usually associated with soil carbon sequestration (Suberkropp and Weyers, 1996; Jastrow et al., 2007) due to their assistance in formation and stabilization of soil aggregates (Strickland and Rousk, 2010). Fungi have been shown to increase aggregation processes by physically

entangling soil particles (Guggenberger et al., 1999; Six et al., 2006) and chemically binding particles with fungal byproducts (Guggenberger et al., 1999; Pikul et al., 1999). Fungal dominance, however, is only one of many factors that affect soil aggregation and is not a necessity in stabilizing aggregates (Wilson et al., 2009), as is demonstrated by our results. Crop residue inputs likely benefited free living, heterotrophic bacteria and saprophytic fungi more so than AMF. When growing in a nutrient rich environment, crops may not rely on beneficial symbiotic relationships such as those with AMF (Janos, 2007), thereby reducing C exudates and minimizing the competitive advantage AMF may have had over bacteria or saprophytic fungi with minimal crop residue input. In addition, bacterial biomass increased with crop residue input, which may imply that the microbial community shifted to a community that is less capable of decomposing more recalcitrant C sources (Ramirez et al., 2012). Although bacterial biomass increased with crop residue input, however, total microbial biomass still decreased. Therefore, the quantity of microbial byproducts likely decreased as well, meaning the change in microbial diversity or biomass did not contribute to aggregate dynamics.

Silt and Clay Dynamics

Silt and clay fractions that become trapped inside aggregates (inter- and intra-SC) experience a temporary change in environmental conditions, which may have contributed to the increase in C concentrations that was seen with increasing crop residue inputs (Figure 2.7). Results show that the storage capacity of SC can be manipulated with a change in environmental conditions, and can be manipulated to a higher degree when trapped in aggregates with higher quality POM

fractions. Free-SC did not differ among crop residue inputs, suggesting this fraction is C saturated.

These results are interesting in that they reveal slight differences between the theoretical two-pool saturation model and actual SOC storage processes. Silt and clay-associated C within the two-pool saturation model is considered a protected pool, and therefore should display a fixed C concentration level once saturated. This study, however, shows that if SC becomes trapped within aggregates the protected pool shows the ability to store additional SOC, meaning the protected pool was no longer C saturated. The C concentration increase, however, is only temporary because once released, SC-associated C will be mineralized until concentrations are equivalent to that of the storage capacity of free-SC. More than likely, the SC fractions are not in a fixed state of C saturation, and the C concentration more accurately is a moving average around the saturation level of free-SC.

Conclusions

Since SOC did not increase with increasing crop residue inputs, and residue decomposition likely did not increase due to N fertilization, soils appear to be C saturated. This was confirmed by the increase of the unprotected pool with the increase in crop residue input, which agrees with the two-pool saturation model (Stewart et al., 2007). It was apparent that N fertilization most strongly affected cPOM quality. After POM is degraded to fPOM and fiPOM, C:N ratios become more stable and are more representative of the level of degradation than initial C:N ratios. The quality of cPOM may have contributed to an increase in the formation of inter-m

within LM, because higher quality cPOM would decompose more rapidly to fiPOM, thereby quickly forming inter-m. In addition, silt and clay trapped within aggregates with higher quality cPOM displayed increased C concentrations among inter- and intra-SC fractions. This study shows C saturation prevented the addition of SOC despite increases in crop residue input, and even though N fertilization most strongly affected cPOM quality, SOC may have been shifted to more protected pools, such as fiPOM, inter- and intra-SC fractions.

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Table 2.1. Means and standard errors of general soil properties. Grain yield is an average of 1999-2011 yield data. All other data is from 2011.

N-application kg ha ⁻¹ y ⁻¹	Grain Yield Mg ha ⁻¹ y ⁻¹	Total Carbon g C kg soil ⁻¹	Total Nitrogen g N kg soil ⁻¹	Whole Soil C:N	Bulk Density g cm ⁻³	Texture - Sand g 100 g soil ⁻¹	Texture - Silt g 100 g soil ⁻¹	Texture - Clay g 100 g soil ⁻¹
0	3.60 ±0.24	17.97 ±0.72	1.53 ±0.04	11.76 ±0.22	1.07 ±0.04	38.58 ±1.65	35.92 ±0.52	25.50 ±1.18
67	6.96 ±0.04	19.10 ±1.77	1.62 ±0.12	11.79 ±0.46	1.08 ±0.04	36.15 ±2.21	37.37 ±1.61	26.48 ±0.79
135	9.01 ±0.13	19.36 ±0.89	1.65 ±0.11	11.77 ±0.32	1.00 ±0.04	37.47 ±1.36	36.42 ±1.16	26.11 ±1.27
202	9.59 ±0.09	20.53 ±1.37	1.64 ±0.10	12.52 ±0.37	1.06 ±0.02	36.37 ±3.14	37.83 ±2.07	25.80 ±1.14
269	9.94 ±0.11	19.80 ±1.03	1.67 ±0.08	11.87 ±0.30	1.13 ±0.03	37.28 ±2.25	36.14 ±0.84	26.58 ±1.52

Table 2.2. Means and standard errors for the total mass proportion aggregate classes and free silt and clay of the whole soil. There were no significant differences among N applications for proportions of any aggregate class or free silt and clay.

N-application kg ha ⁻¹ y ⁻¹	Large Macros g 100 g ⁻¹ soil	Small Macros g 100 g ⁻¹ soil	Free-micros g 100 g ⁻¹ soil	Free-SC g 100 g ⁻¹ soil
0	1.43 ±0.14	28.16 ±1.34	22.40 ±0.73	8.95 ±0.59
67	1.19 ±0.19	31.32 ±2.09	21.23 ±3.84	6.95 ±0.56
135	2.34 ±0.65	29.57 ±3.22	22.36 ±2.50	7.91 ±0.57
202	2.46 ±1.23	28.39 ±2.27	24.79 ±1.63	8.04 ±0.29
269	3.31 ±1.30	25.90 ±1.54	23.68 ±0.87	9.17 ±0.70

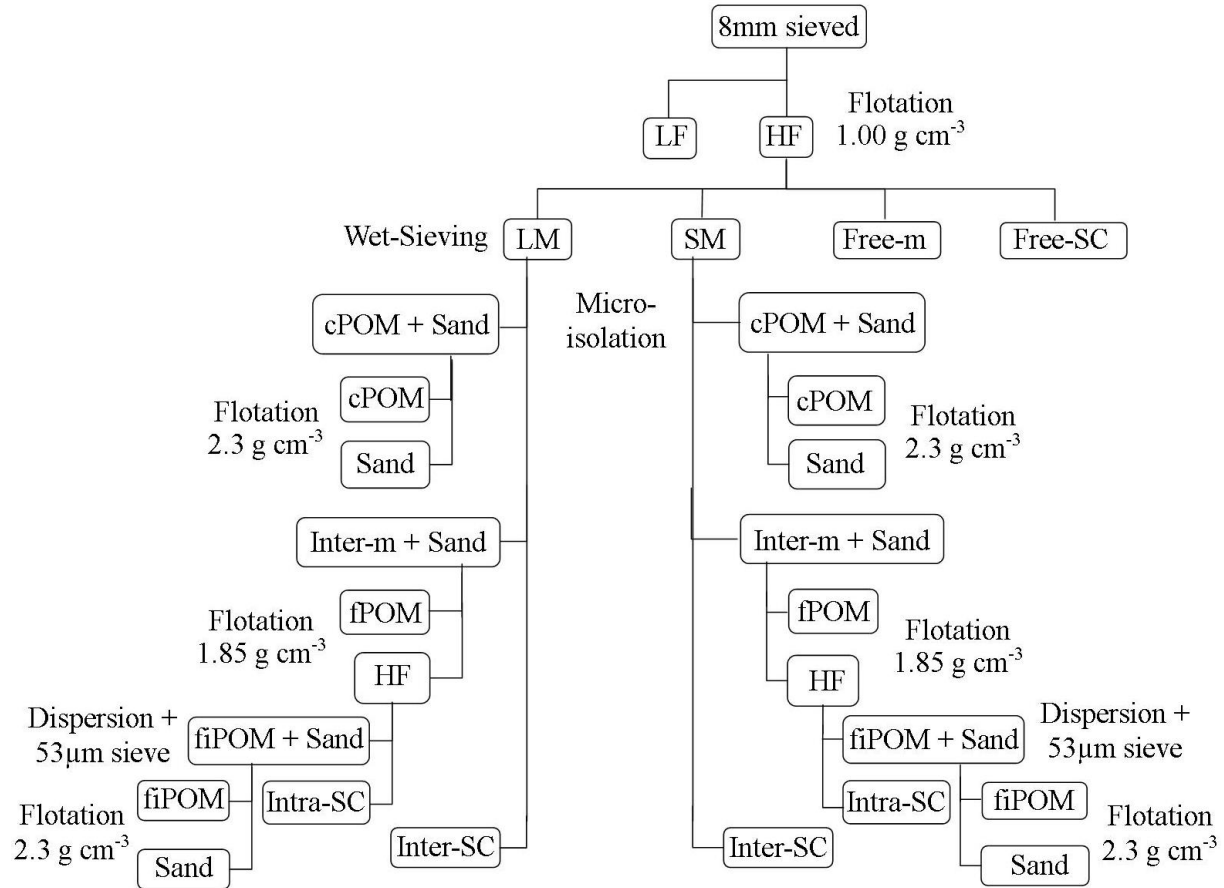


Figure 2.1. Laboratory soil organic matter fractionation. The light fraction (LF) was removed from 8mm sieved whole soil with density flotation. The heavy fraction (HF) was then wet-sieved to fractionate water stable aggregate classes (LM=Large Macros, SM=Small Macros, Free-m=micros) from silt and clay (Free-SC). Coarse particulate organic matter (cPOM), microaggregates within macroaggregates (Inter-m), and macroaggregate silt and clay (Inter-m) were then isolated from LMs and SMs. A density flotation was used to remove fine particulate organic matter (fPOM) before microaggregate dispersion. Dispersion of the Inter-m mixture then released fine intra-aggregate particulate organic matter fiPOM and silt and clay that was within Inter-m (Intra-SC). Density flotations were also used to remove any sand contamination.

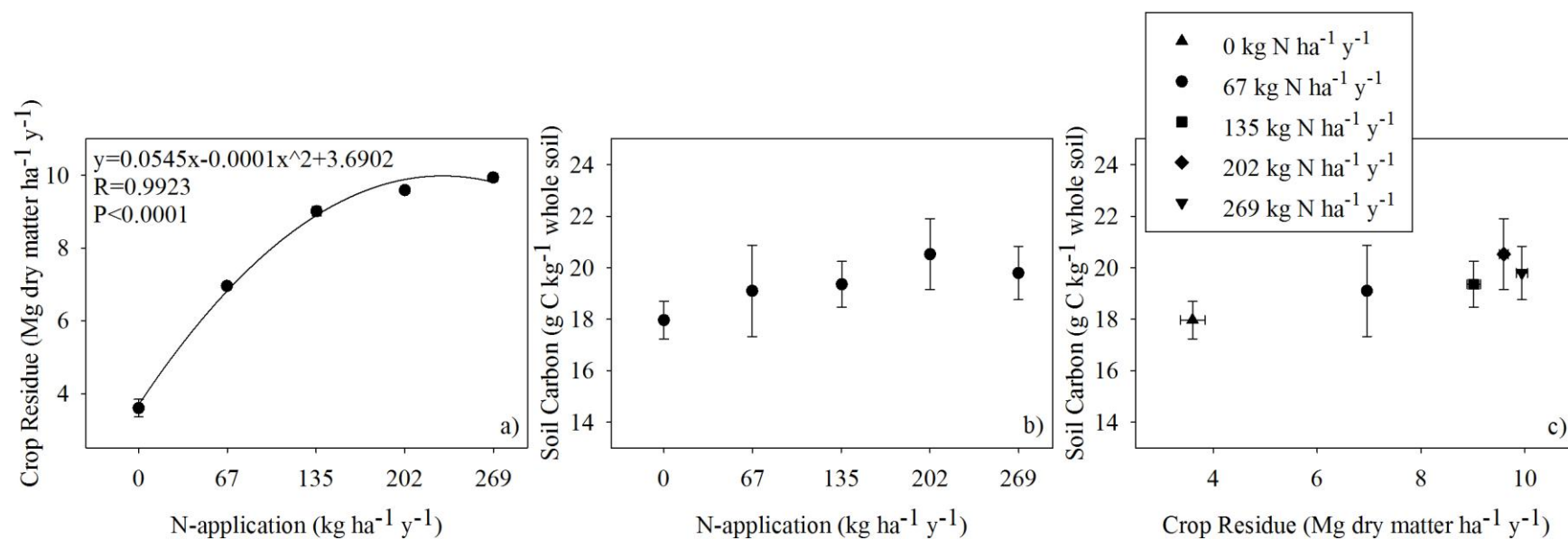


Figure 2.2. a) Crop residue as a function of N application. Agronomic optimum nitrogen rate (AONR) was calculated at 208.4 kg N ha⁻¹ and yielded 9.78 Mg grain ha⁻¹. b) Whole soil carbon concentration was not affected by N application rate or c) crop residue input. All error bars represent standard errors.

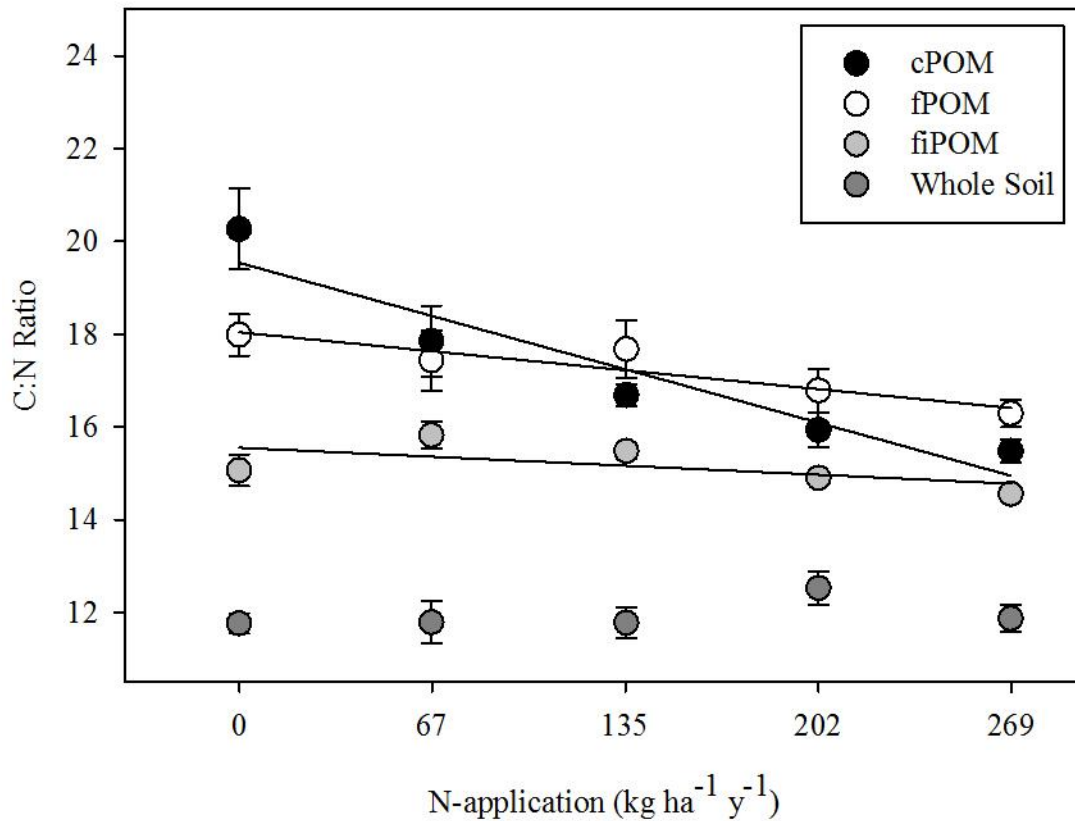


Figure 2.3. C:N ratios for POM fractions within small plus large macroaggregates. Significant linear relationships were found with nitrogen application rate and cPOM ($P < 0.0001$, $R = 0.7125$), fPOM ($P = 0.0079$, $R = 0.4481$), and fiPOM ($P = 0.0220$, $R = 0.3614$). Regression equations for cPOM, fPOM, and fiPOM were: $y = -0.0171x + 19.5380$, $y = -0.0061x + 18.0384$, and $y = -0.0029x + 15.5509$, respectively and the regression slope coefficients significantly differed between cPOM and fPOM ($P = 0.0027$) and between cPOM and fiPOM ($P < 0.0001$). Error bars represent standard errors.

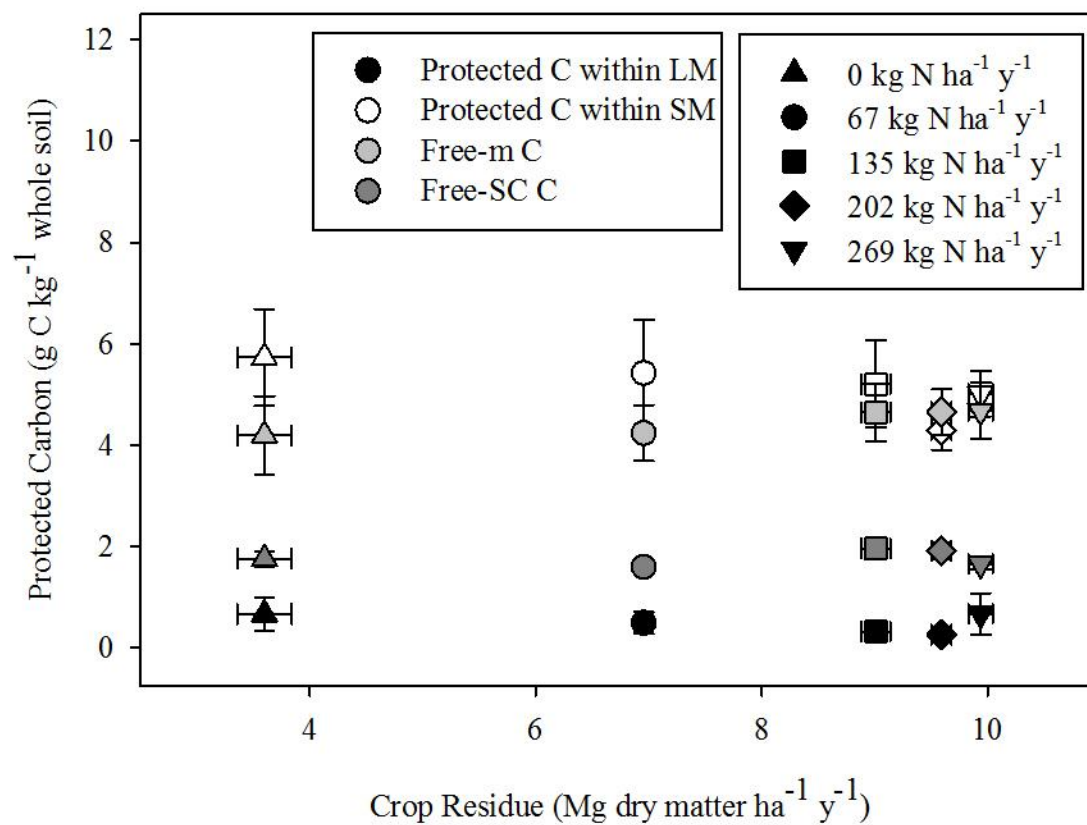


Figure 2.4. There was no significant effect of crop residue on protected C fractions. Values represent protected carbon within macroaggregates (the sum of C from inter-m, inter-SC, and intra-SC), protected carbon within microaggregates (the sum of free-m and intra-SC), and free protected C (free-SC). Error bars represent standard errors.

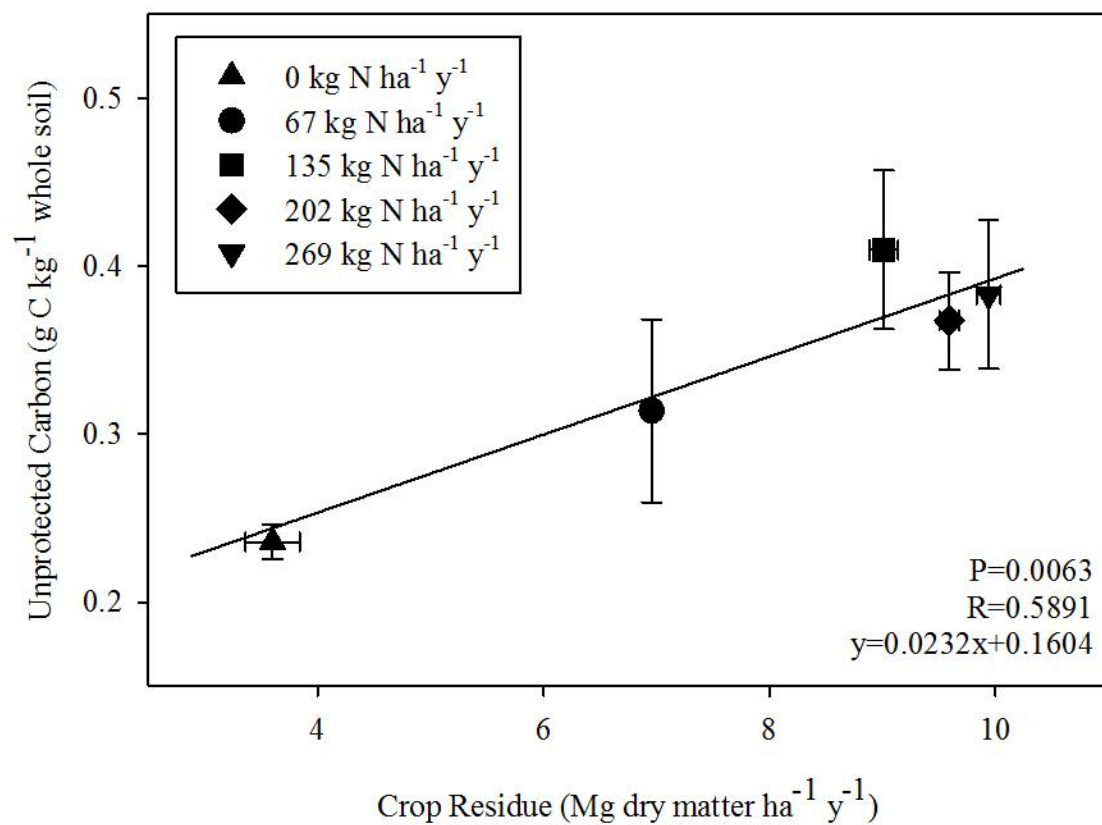


Figure 2.5. Unprotected carbon (sum of cPOM and fPOM) was positively correlated with crop residue input. Error bars represent standard errors.

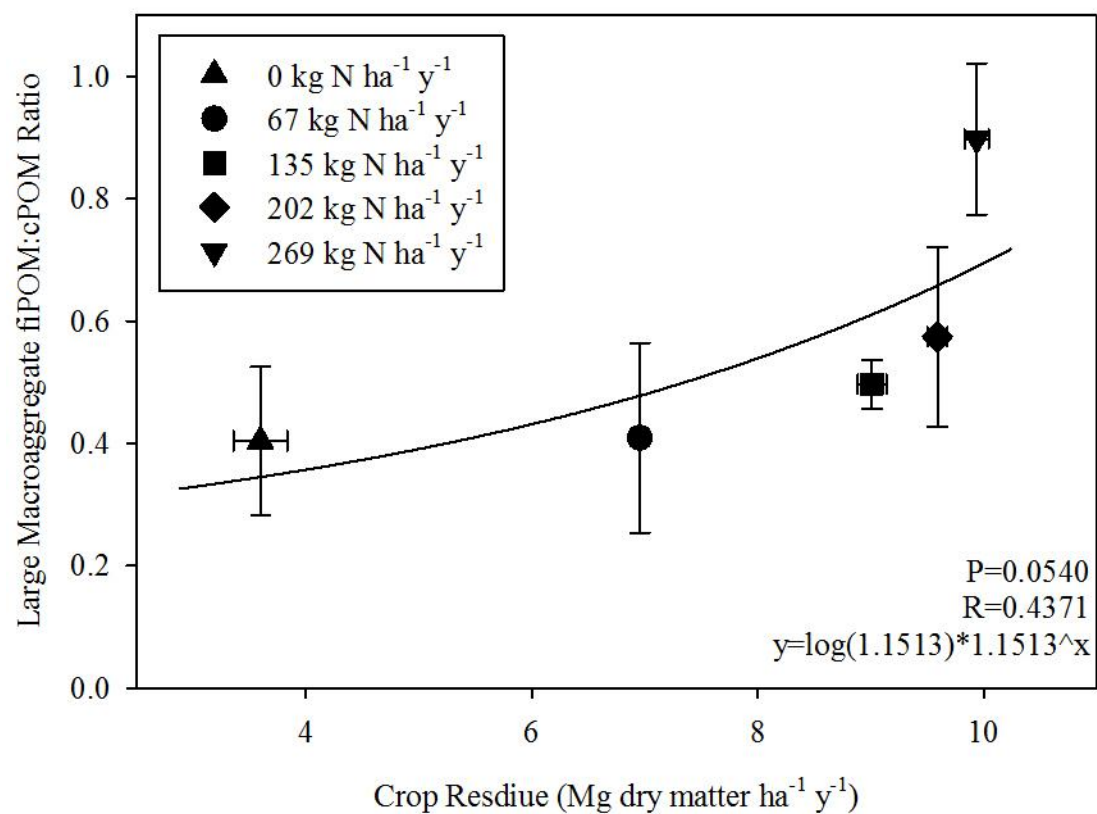


Figure 2.6. The ratio of fiPOM:cPOM in large macroaggregates increased with crop residue input. Error bars represent standard errors.

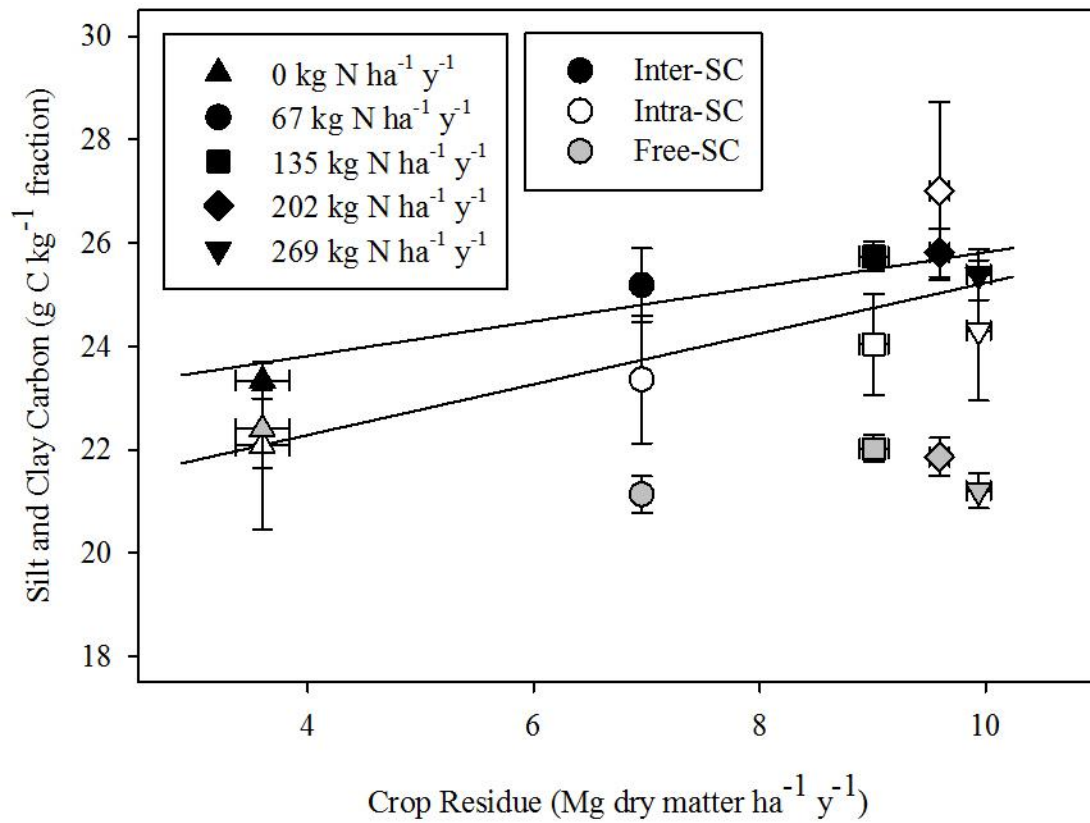


Figure 2.7. Carbon concentration of free silt and clay (free-SC) fractions as well as silt and clay fractions trapped within aggregates (Inter- and Intra-SC). There were significant relationships between crop residue and Inter-SC ($P=0.0009$, $R=0.5051$) and slightly significant relationships between crop residue and Intra-SC ($P=0.0745$, $R=0.2851$). Regression equations for Inter-SC and Intra-SC were: $y=0.3346x+22.4724$ and $y=0.4909x+20.3169$, respectively. Free-SC was not related to crop residue input. Error bars represent standard errors.

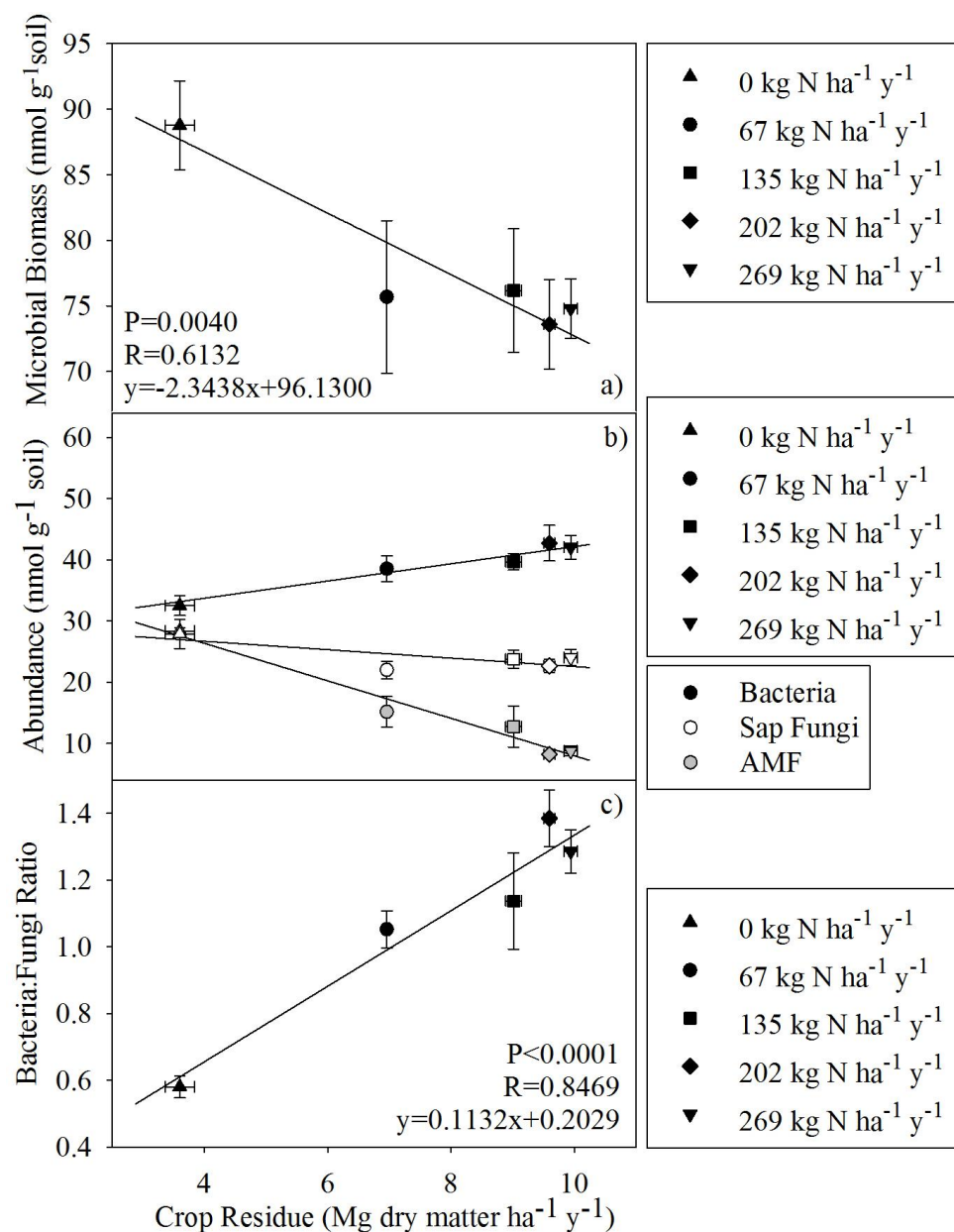


Figure 2.8. a) Microbial biomass (sum of bacteria, saprophytic fungi (sap fungi), and arbuscular mycorrhizal fungi (AMF) lipid abundance) decreased with crop residue input. b) Lipid abundance for bacteria, sap fungi, and AMF. Crop residue was positively correlated with bacteria ($P=0.0018$, $R=0.6531$) and negatively correlated with saprophytic fungi ($P=0.0363$, $R=-0.4704$), and AMF ($P<0.0001$, $R=-0.8971$). Regression equations for bacteria, sap fungi, and AMF were: $y=1.4064x+28.1000$, $y=-0.6873x+29.4345$, and $y=-3.0630x+38.5955$, respectively. c) Bacteria:Fungi (sap fungi + AMF) ratio increased with crop residue input. Error bars represent standard errors.

CHAPTER 3. GENERAL CONCLUSIONS

It is highly debated if N fertilization causes a positive or negative effect on SOC when applied on corn-based cropping systems. The research presented in this thesis observed SOC storage dynamics of a continuous corn system that has received five rates of N fertilization for 13 years in Iowa, USA.

Results from this study (presented in Chapter 2) indicated that there was little-to-no change in SOC despite increases in crop residue inputs. Protected pools did not differ, whereas unprotected pool increased linearly with crop residue inputs, indicating that soils were C saturated and follow a response typical with the two-pool saturation model. Nitrogen fertilization did not affect the quality of crop residue input beyond cPOM, and after being degraded to fPOM, the range of C:N ratios became very narrow across N application rates. The increase in cPOM quality may have altered aggregate dynamics by quickly decomposing high quality cPOM to fiPOM and, in turn, quickly forming inter-m. Soils with increased crop residue input and increased cPOM quality showed an increase in fiPOM:cPOM ratio among LM, but the proportion of LM did not increase. This may imply that the increase in inter-m formation may happen without an increase in LM stabilization. In addition, silt and clay fractions that remained trapped inside aggregates with decomposing high quality cPOM experienced increases in C storage capacities. This study shows that SOC did not increase despite increases in crop residue input due to C saturation. It also shows that through N fertilization, an increase in cPOM quality may have shifted SOC to more stable soil fractions, such as fiPOM, inter- and intra-SC fractions.

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